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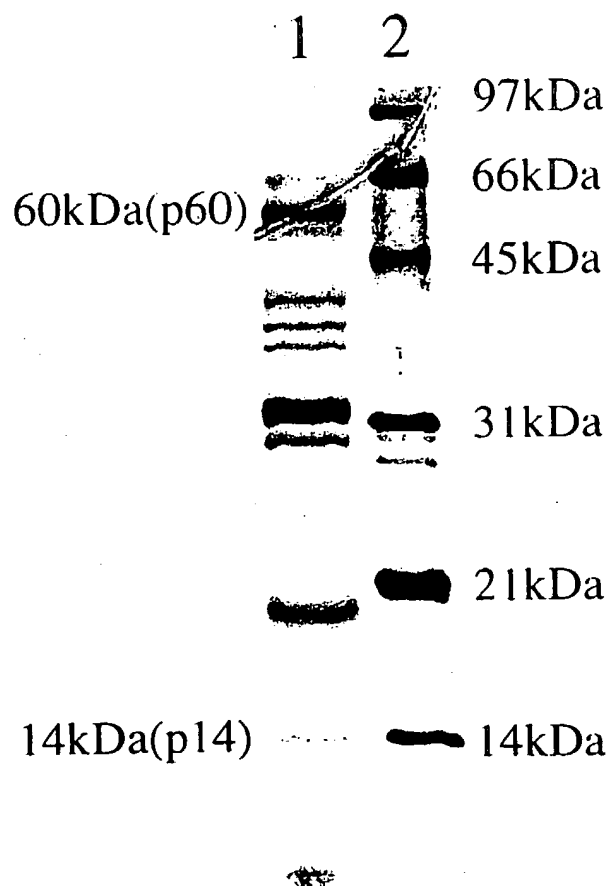
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FIG. 1



Purification Result for Endo- β -N-acetylglucosaminidase (15-25% gradient SDS-PAGE)

Lane 1: Purified endo- β -N-acetylglucosaminidase from *Mucor hiemalis*

Lane 2: Molecular weight markers

FIG. 3

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10      20      30      40      50      60
GTCGACCCAC GCGTCCCGCG ACGCGTGGGC GGACCGGTGG GCGGACCGGT GGGTTTTATT

70      80      90      100     110     120
TTACATAAAT ATGCCTTCAC TTCAATTGCA ACCTGATGAC AAACCTAGCAC CTGTTTCTTT

130     140     150     160     170     180
TGCACTTAAG TCTATGAATG AGTTGAGGGA CTGGACGCCA GACGAAAAGA TAAAGTTTAA

190     200     210     220     230     240
CGTTTCAAGC GTGGCACTAC AGCCTCGTGT GAAAAACGCC CTGAAACCTC AATTATTTGT

250     260     270     280     290     300
AACTCATGAT ATGGCAGGAG GATATAAAGA AGATAAAAT ATTCAAGGAA ACAATTATAA

310     320     330     340     350     360
AGACATTTAT AACATTCAAT ATTGGCATT TTGTGATATT TTTGTATATT TCTCTCATGA

370     380     390     400     410     420
GCGAGTTAGC ATTCTCCAG TCAATTGGAC AAATGCTTGT CATAGAAATG GTGTAAAGTG

430     440     450     460     470     480
TTTAGGTACT TTTTGTAGTAG AAGGAAATAA CCAAATGCAT GAAATGGAAG CCTTGCTTCA

490     500     510     520     530     540
CGGTCCACCT TTAATTAAATA AACTGACGA CCTATGAGA TTATGGAGTC CGTATTATGC

550     560     570     580     590     600
AGACCAATTA GTTGCTATTG CTAAACACTA TGGTTTGTAT GGCTGGTTGT TCAATATTGA

610     620     630     640     650     660
ATCGGAATTC TTTCTTTTC CTACAAATCC AAAATTCAA GCTGAAGAGT TGGCAAAGTT

670     680     690     700     710     720
TCTACACTAT TTTAAGGAAA AATTGCATAA CGAAATACCT GGATCTCAAC TCATTGTGTA

730     740     750     760     770     780
CGACAGCATG ACAAATGAAG GAGAAATCCA CTGGCAGAAC CAGCTCACAT GGAAAAATGA

790     800     810     820     830     840
GTEATTTTTT AAAAACACGG ATGGTATTTT TTTGAATTAT TGGTGGAAAA AAGAATACCC

850     860     870     880     890     900
TGAAATGGCG CGTAGAGTAG CTGAAGGAAT AGGTAGATCA GGTTTAGAAG TTTATTTTGG

910     920     930     940     950     960
TACAGATGTA TGGGAAGGC ATACTTATGG TGGCGTGGT TTCAAATCAT ATAAGGGTGT

970     980     990     1000    1010    1020
AAAAACTGCC TACTCTGCAA TGACATCTTC TGCATTATTT GGTATGGCAT GGACATACGA

1030    1040    1050    1060    1070    1080
GCATTTCGAA AAGTCTGAAT TTGAAAGAT GGATCGTTTG TTTTGGTGTG GTGGTAAATA

1090    1100    1110    1120    1130    1140
CTCTGACTAT CCTCCCCAC CTCTTAAAAA CCCAGATGAC GAAAAAGAAG TAGAAAGCGA

1150    1160    1170    1180    1190    1200
TCATAGTGAA GATGAGCTCA TGTACGGACA CAAGAAAGGT ATTGCTGACA CGGTAGAATC

1210    1220    1230    1240    1250    1260
TATTCCTGTA CCAGGAACAG ATTGGTTTGT TACCAATTTT GATAGGGGGT TTGGAATAG

1270    1280    1290    1300    1310    1320
GTTTTATTAT AGAGGAAAGA GATTACTTTC TCAGCCTTGG TCCCATTTAT CGCATCAAGC

1330    1340    1350    1360    1370    1380
TATTCCTCCC AATAAAAGCT ATCGAAATCC AGAGATTAT CCCACTGATC AAAACATTAA

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Entire nucleotide sequence of the fragment inserted into the Sal I-Not I sites of pZL-Endo including the full-length novel endo- β -N-acetylglucosaminidase gene

FIG. 4

1390	1400	1410	1420	1430	1440
AATCACTAGT	TCTCTCGATT	GCGATCATGG	AGCTTTTCTT	GGTGGAACTT	CGCTTATTAT
1450	1460	1470	1480	1490	1500
CAAAGGCCAA	CGTTTCAATC	ATAGAGAATC	GCATGATGTT	GAAACTGAAA	TTAGTATACC
1510	1520	1530	1540	1550	1560
TCTGTATAAG	CTTTCATTAG	ATGCTAGTAA	AGGATGCTCA	TTGCGTTATA	TTTATAGAAC
1570	1580	1590	1600	1610	1620
TTTGTGATG	AAAGATGTAA	AGTTGACAGT	AGCATGTCAC	TTTTCGTTAA	AAACAAACGA
1630	1640	1650	1660	1670	1680
CTCAGTTAAT	TTCTTCAAGG	TATGGCAGCC	AGATGAAAAT	TTCTCTTTTG	AATATGATGA
1690	1700	1710	1720	1730	1740
TGGAATGAGA	GCCACTGTTA	CAACTGAAAA	TTCTACCGAA	AGCAGATGCT	TTTTATTACG
1750	1760	1770	1780	1790	1800
TACAACAGAA	GAAGATACAG	GAGAAAATGA	TTGGATAACA	AAAACATTAA	ATGTGCCTGC
1810	1820	1830	1840	1850	1860
TGTTCCAGAA	GGAAGTCAAT	TATACATTAC	AAGACTTGAA	GTGAGCGTAG	TATTAGATAC
1870	1880	1890	1900	1910	1920
AGCTGGTTTA	GTAGGTCTTG	TTAATCAAGT	TATTGCTTGC	TTGGGATATA	TTAGCATCAT
1930	1940	1950	1960	1970	1980
ACCAACTATA	AATTCTGGAA	TAAAAACAGA	TTATTCACGC	ATTATTTCAGG	ATCTCTTTTG
1990	2000	2010	2020	2030	2040
GAAAGATCAG	AAATATACCA	AAATCGGAAA	AGAAAGTTTA	GACGACATAG	CTCAAGAAGA
2050	2060	2070	2080	2090	2100
AGTTCATAGA	TATTATGGAA	CATTGAACTG	GGAAAACACA	GCAAATGTAG	TAAACGCTTG
2110	2120	2130	2140	2150	2160
GGAGGAAATA	GATTACTACA	ACGTTTTTTA	CAAAGAAAGT	GACGACTCTG	CAACTCGCAT
2170	2180	2190	2200	2210	2220
CTTTTATAGGA	ACAGCATTCT	GTAATCAATT	TCGTGTATCT	GGTTTAGATA	TTATTTTATC
2230	2240	2250	2260	2270	2280
TAAGCTACCA	AAGATAGTTA	TTGAAGCTGT	TAACAAAGAA	GGATACATCT	CTTCAAGTGG
2290	2300	2310	2320	2330	2340
TAGCATAGAT	TTGTCAATTAA	ACTAGGACTT	GAAATAAAAT	ATTATGATAA	AGAAAAAATA
2350	2360	2370	2380	2390	2400
AAAAAAAAAA	AAAAAAAAAG	GGCGGCCGC.

Entire nucleotide sequence of the fragment inserted into the Sal I-Not I sites of pZL-Endo including the full-length novel endo- β -N-acetylglucosaminidase gene (Continued)

FIG. 5

5'	ATG	CCT	TCA	CTT	CAA	TTG	CAA	CCT	GAT	GAC	AAA	CTA	GCA	CCT	GTT	TCT	TTT	GCA	54
	M	P	S	L	Q	L	Q	P	D	D	K	L	A	P	V	S	P	A	
	CTT	AAG	TCT	ATG	AAT	GAG	TTG	AGG	GAC	TGG	ACG	CCA	GAC	GAA	AAG	ATA	AAG	TTT	108
	L	K	S	M	N	E	L	R	D	W	T	P	D	E	K	I	K	F	
	AAC	GTT	TCA	AGC	GTG	GCA	CTA	CAG	CCT	CGT	GTG	AAA	AAC	GCC	CTG	AAA	CCT	CAA	162
	N	V	S	S	V	A	L	Q	P	R	V	K	N	A	L	K	P	Q	
	TTA	TTG	TTA	ACT	CAT	GAT	ATG	GCA	GGA	GGA	TAT	AAA	GAA	GAT	AAA	AAT	ATT	CAA	216
	L	L	L	T	H	D	M	A	G	G	Y	K	E	D	K	N	I	Q	
	GGA	AAC	AAT	TAT	AAA	GAC	ATT	TAT	AAC	ATT	CAA	TAT	TGG	CAT	TTA	GCT	GAT	ACT	270
	G	N	N	Y	K	D	I	Y	N	I	Q	Y	W	H	L	A	D	T	
	TTT	GTA	TAT	TTC	TCT	CAT	GAG	CGA	GTT	AGC	ATT	CCT	CCA	GTC	AAT	TGG	ACA	AAT	324
	F	V	Y	F	S	H	E	R	V	S	I	P	P	V	N	W	T	N	
	GCT	TGT	CAT	AGA	AAT	GGT	GTA	AAG	TGT	TTA	GGT	ACT	TTT	TTA	GTA	GAA	GGA	AAT	378
	A	C	H	R	N	G	V	K	C	L	G	T	F	L	V	E	G	N	
	AAC	CAA	ATG	CAT	GAA	ATG	GAA	GCC	TTG	CTT	CAC	GGT	CCA	CCT	TTA	CTT	AAT	AAC	432
	N	Q	M	H	E	M	E	A	L	L	H	G	P	P	L	L	N	N	
	ACT	GAC	GAC	CCT	ATG	AGA	TTA	TGG	AGT	CCG	TAT	TAT	GCA	GAC	CAA	TTA	GTT	GCT	486
	T	D	D	P	M	R	L	W	S	P	Y	Y	A	D	Q	L	V	A	
	ATT	GCT	AAA	CAC	TAT	GGT	TTT	GAT	GGC	TGG	TTG	TTC	AAT	ATT	GAA	TGC	GAA	TTC	540
	I	A	K	H	Y	G	F	D	G	W	L	F	N	I	E	C	E	F	
	TTT	CCT	TTT	CCT	ACA	AAT	CCA	AAA	TTC	AAA	GCT	GAA	GAG	TTG	GCA	AAG	TTT	CTA	594
	F	P	F	P	T	N	P	K	F	K	A	E	E	L	A	K	F	L	
	CAC	TAT	TTT	AAG	GAA	AAA	TTG	CAT	AAC	GAA	ATA	CCT	GGA	TCT	CAA	CTC	ATT	TGG	648
	H	Y	F	K	E	K	L	H	N	E	I	P	G	S	Q	L	I	W	
	TAC	GAC	AGC	ATG	ACA	AAT	GAA	GGA	GAA	ATC	CAC	TGG	CAG	AAC	CAG	CTC	ACA	TGG	702
	Y	D	S	M	T	N	E	G	E	I	H	W	Q	N	Q	L	T	W	

Amino acid sequence deduced from the novel Endo- β -N-acetylglucosaminidase gene, and the nucleotide sequence of the DNA encoding this amino acid sequence.

FIG. 6

AAA	AAT	711	GAG	TTA	TTT	720	TTT	AAA	AAC	729	ACG	GAT	GGT	738	ATT	TTT	TTG	747	AAT	TAT	TTG	756	TTG
K	N	E	L	F	F	K	N	T	D	G	I	F	L	N	Y	W	W						
AAA	AAA	765	GAA	TAC	CCT	774	GAA	ATG	GCG	783	CGT	AGA	GTA	792	OCT	GAA	GGA	801	ATA	GGT	AGA	810	TCA
K	K	E	Y	P	E	M	A	R	R	V	A	E	G	I	G	R	S						
GGT	TTA	819	GAA	GTT	TAT	828	TTT	GGT	ACA	837	GAT	GTA	TGG	846	GGA	AGG	CAT	855	ACT	TAT	GGT	864	GGC
G	L	E	V	Y	F	G	T	D	V	W	G	R	H	T	Y	G	G						
GGT	GGT	873	TTC	AAA	TCA	882	TAT	AAG	GGT	891	GTA	AAA	ACT	900	GCC	TAC	TCT	909	GCA	ATG	ACA	918	TCT
G	G	F	K	S	Y	K	G	V	K	T	A	Y	S	A	M	T	S						
TCT	GCA	927	TTA	TTT	GGT	936	ATG	GCA	TGG	945	ACA	TAC	GAG	954	CAT	TTC	GAA	963	AAG	TCT	GAA	972	TTT
S	A	L	F	G	M	A	W	T	Y	E	H	F	E	K	S	E	F						
GAA	AAG	981	ATG	GAT	CGT	990	TTG	TTT	TGG	999	TGT	GGT	GGT	1008	AAA	TAC	TCT	1017	GAC	TAT	CCT	1026	CCC
E	K	M	D	R	L	F	W	C	G	G	K	Y	S	D	Y	P	P						
CCA	CCT	1035	CCT	AAA	AAC	1044	CCA	GAT	GAC	1053	GAA	AAA	GAA	1062	GTA	GAA	AGC	1071	GAT	GAT	AGT	1080	GAA
P	P	P	K	N	P	D	D	E	K	E	V	E	S	D	D	S	E						
GAT	GAG	1089	CTC	ATG	TAC	1098	GGA	CAC	AAG	1107	AAA	GGT	ATT	1116	GCT	GAC	ACG	1125	GTA	GAA	TCT	1134	ATT
D	E	L	M	Y	G	H	K	K	G	I	A	D	T	V	E	S	I						
CCT	GTA	1143	CCA	GGA	ACA	1152	GAT	TGG	TTT	1161	GTT	ACC	AAT	1170	TTT	GAT	AGG	1179	GGG	TTT	GGA	1188	AAT
P	V	P	G	T	D	W	F	V	T	N	F	D	R	G	F	G	N						
AGG	TTT	1197	TAT	TAT	AGA	1206	GGA	AAG	AGA	1215	TTA	CTT	TCT	1224	CAG	OCT	TGG	1233	TCC	CAT	TTA	1242	TGG
R	F	Y	Y	R	G	K	R	L	L	S	Q	P	W	S	H	L	S						
CAT	CAA	1251	GCT	ATT	CTC	1260	CCC	AAT	AAA	1269	AGC	TAT	CGA	1278	AAT	CCA	GAG	1287	ATT	TAT	CCC	1296	ACT
H	Q	A	I	L	P	N	K	S	Y	R	N	P	E	I	Y	P	T						
GAT	CAA	1305	AAC	ATT	AAA	1314	ATC	ACT	AGT	1323	TCT	CTC	GAT	1332	TGC	GAT	CAT	1341	GGA	GCT	TTT	1350	CTT
D	Q	N	I	K	I	T	S	S	L	D	C	D	H	G	A	F	L						
GGT	GGA	1359	ACC	TCG	CTT	1368	ATT	ATC	AAA	1377	GGC	CAA	CGT	1386	TTC	AAT	CAT	1395	AGA	GAA	TGG	1404	CAT
G	G	T	S	L	I	I	K	G	Q	R	F	N	H	R	E	S	H						
GAT	GTT	1413	GAA	ACT	GAA	1422	ATT	AGT	ATA	1431	CCT	CTG	TAT	1440	AAG	CTT	TCA	1449	TTA	GAT	GCT	1458	AGT
D	V	E	T	E	I	S	I	P	L	Y	K	L	S	L	D	A	S						

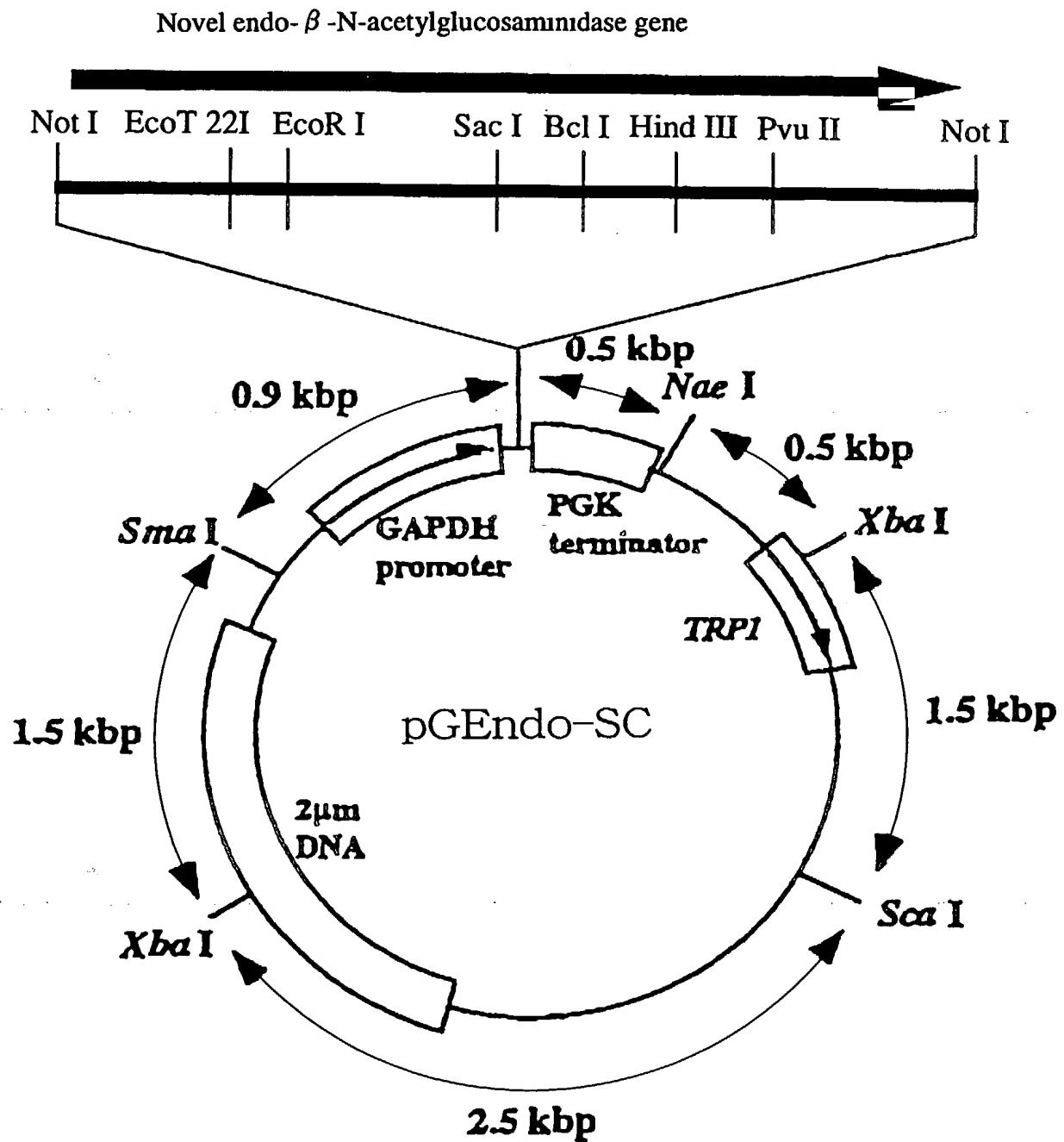
Amino acid sequence deduced from the novel Endo- β -N-acetylglucosaminidase gene, and the nucleotide sequence of the DNA encoding this amino acid sequence (Continued)

FIG. 7

1467	1476	1485	1494	1503	1512
AAA GGA TOC TCA TTG CGT TAT ATT TAT AGA ACT TTG TTG ATG AAA GAT GTA AAG					
K G C S L R Y I Y R T L L H K D V K					
1521	1530	1539	1548	1557	1566
TTG ACA GGA GCA TGT CAC TTT TCG TTA AAA ACA AAC GAC TCA GTT AAT TTC TTC					
L T V A C H F S L K T N D S V N F P					
1575	1584	1593	1602	1611	1620
AAG GTA TGG CAG CCA GAT GAA AAT TTC TCT TTT GAA TAT GAT GAT GGA ATG AGA					
K V W Q P D E N F S F E Y D D G H R					
1629	1638	1647	1656	1665	1674
GCC ACT GTT ACA ACT GAA AAT TCT ACC GAA AGC AGA TGC TTT TTA TTA CGT ACA					
A T V T T E N S T E S R C F L L R T					
1683	1692	1701	1710	1719	1728
ACA GAA GAA GAT ACA GGA GAA AAT GAT TGG ATA ACA AAA ACT ATT AAT GTG CCT					
T E E D T G E N D W I T K T I N V P					
1737	1746	1755	1764	1773	1782
GCT GTT CCA GAA GGA AGT CAA TTA TAC ATT ACA AGA CTT GAA GTG AGC GTA GTA					
A V P E G S Q L Y I T R L E V S V V					
1791	1800	1809	1818	1827	1836
TTA GAT ACA GCT GGT TTA GTA GGT CTT GTT AAT CAA GTT ATT GCT TGC TTG GGA					
L D T A G L V G L V N Q V I A C L G					
1845	1854	1863	1872	1881	1890
TAT ATT AGC ATC ATA CCA ACT ATA AAT TCT GGA ATA AAA ACA GAT TCA TCA CGC					
Y I S I I P T I N S G I K T D S S R					
1899	1908	1917	1926	1935	1944
ATT ATT CAG GAT CTC TTT TGG AAA GAT CAG AAA TAT ACC AAA ATC GGA AAA GAA					
I I Q D L F W K D Q K Y T K I G K E					
1953	1962	1971	1980	1989	1998
AGT TTA GAC GAC ATA GCT CAA GAA GAA GTT CAT ACA TAT TAT GGA ACA TTG AAC					
S L D D I A Q E E V H R Y Y G T L N					
2007	2016	2025	2034	2043	2052
TGG GAA AAC ACA GCA AAT GTA GTA AAC GCT TGG GAG GAA ATA GAT TAC TAC AAC					
W E N T A N V V N A W E E I D Y Y N					
2061	2070	2079	2088	2097	2106
GTT TTT TAC AAA GAA AGT GAC GAC TCT GCA ACT CGC ATC TTT TTA GGA ACA GCA					
V P Y K E S D D S A T R I F L G T A					
2115	2124	2133	2142	2151	2160
TTC TGT AAT CAA TTT CGT GTA TCT GGT TTA GAT ATT ATT TTA TCT AAG CTA CCA					
F C N Q P R V S G L D I I L S K L P					
2169	2178	2187	2196	2205	2214
AAG ATA GTT ATT GAA GCT GTT AAC AAA GAA GGA TAC ATC TCT TCA AGT GGT AGC					
K I V I E A V N K E G Y I S S S G S					
2223	2232				
ATA GAT TTG TCA TTA AAC TAG 3'					
I D L S L N *					

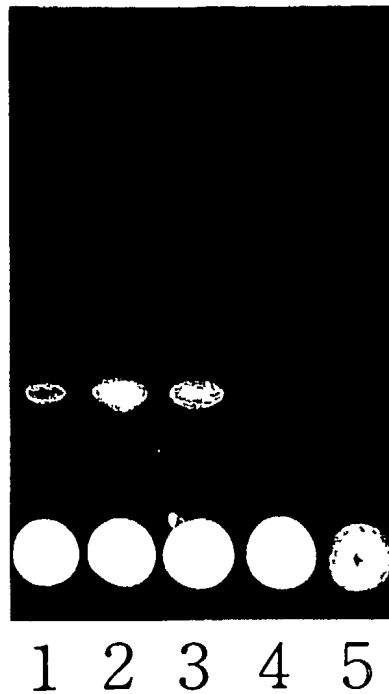
Amino acid sequence deduced from the novel Endo- β -N-acetylglucosaminidase gene, and the nucleotide sequence of the DNA encoding this amino acid sequence (Continued)

FIG. 8



Structure of expression vector pGEndo-SC for the use in *Saccharomyces cerevisiae*, which comprises a novel endo- β -N-acetylglucosaminidase gene

FIG. 9



Expression of endo- β -N-acetylglucosaminidase enzyme in yeast into which
an endo- β -N-acetylglucosaminidase gene has been introduced

Lanes 1-3 Cellular extract of *S. cerevisiae* YPH500 (pep4) into which
an endo- β -N-acetylglucosaminidase gene has been introduced.

Lane 4 Purified endo- β -N-acetylglucosaminidase derived from *M. hiemalis*

Lane 5. Cellular extract of *S. cerevisiae* YPH500 (pep4)